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Suppression of Inhibitory Synaptic Potentiation by Presynaptic Activity through Postsynaptic $GABA_B$ Receptors in a Purkinje Neuron

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Summary

At inhibitory synapses on a cerebellar Purkinje neuron, the depolarization caused by heterosynaptic climbing fiber activation induces long-lasting potentiation accompanied by an increase in $GABA_A$ receptor responsiveness. Here we show that activation of a presynaptic inhibitory interneuron during the conditioning postsynaptic depolarization suppresses the potentiation. The suppression is due to postsynaptic $GABA_B$ receptor activation by GABA released from presynaptic terminals. The results suggest that $GABA_B$ receptor activation decreases the activity of cAMP-dependent protein kinase through the G_i/G_o proteins. The presynaptic activity-dependent suppression of synaptic plasticity is a novel regulatory mechanism of synaptic efficacy at individual synapses and may contribute to the learning and computational ability of the cerebellar cortex.

Introduction

Most forms of synaptic plasticity are triggered by repetitive activation of the presynaptic neurons and have been considered a cellular basis for learning and memory (see Ito, 1989; Bliss and Collingridge, 1993; Malenka, 1994; Kano, 1995; Marty and Llano, 1995; Linden and Connor, 1995; Daniel et al., 1998). For example, long-term potentiation (LTP) in the hippocampus is induced by high-frequency presynaptic activation and the long-term depression (LTD) by low-frequency activation (Bliss and Collingridge, 1993; Malenka, 1994). In the cerebellum, LTD at glutamatergic synapses between parallel fibers and a Purkinje neuron (PN) is induced when the presynaptic parallel fibers are repetitively activated in conjunction with climbing fiber activation (see Ito, 1989; Linden and Connor, 1995; Daniel et al., 1998). The involvement of presynaptic activity in induction of synaptic plasticity then enables independent regulation of transmission efficacy at each synapse. On the other hand, at inhibitory GABAergic synapses between an inhibitory interneuron (IN, stellate, or basket neuron) and a PN, the postsynaptic depolarization caused by heterosynaptic climbing fiber activation results in a long-lasting potentiation known as rebound potentiation (RP) (Kano et al., 1992; also see Kano, 1995). RP is accompanied by increased responsiveness of postsynaptic $GABA_A$ receptors ($GABA_A$Rs) and involves postsynaptic Ca^{2+} and Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) (Kano et al., 1992, 1996; Hashimoto et al., 1996). RP seems to be a postsynaptic phenomenon brought about by heterosynaptic inputs, and its induction does not require the presynaptic activation. Thus, RP is a mechanism that regulates the transmission efficacy at a number of synapses simultaneously. We wondered that the IN activity may also play a role in RP and have examined the effect of presynaptic activation on RP induction. We show here that activation of a presynaptic IN during the conditioning postsynaptic depolarizations suppresses RP, and we suggest that this mechanism enables synapse-specific regulation of transmission efficacy. Further, we have investigated the molecular mechanism of the suppression of RP and have demonstrated the role of postsynaptic $GABA_B$ receptors ($GABA_B$Rs).

$GABA_B$Rs are G protein–coupled metabotropic receptors localized at both presynaptic and postsynaptic sites (Kaupmann et al., 1997, 1998; Jones et al., 1998; White et al., 1998; Kuner et al., 1999). Presynaptic $GABA_B$Rs regulate transmitter release, and postsynaptic $GABA_B$Rs activate K^+ conductance and induce slow inhibitory postsynaptic potential (IPSP) (Newberry and Nicoll, 1984; Dutar and Nicoll, 1988; Batchelor and Garthwaite, 1992; Jarolimek and Misgeld, 1997; also see Misgeld et al., 1995). $GABA_B$Rs inhibit adenylyl cyclase through activation of the G_i/G_o proteins (Misgeld et al., 1995; Kaupmann et al., 1997; Kuner et al., 1999). Two cases implicating the role of $GABA_B$Rs in synaptic plasticity have been reported. One is the role of presynaptic $GABA_B$Rs in the regulation of hippocampal LTP (Davies et al., 1991; Mott and Lewis, 1991), and the other is the role of postsynaptic $GABA_B$Rs in LTP induction at inhibitory synapses in the visual cortex (Komatsu, 1996). In the latter case, it was suggested that activation of postsynaptic $GABA_B$Rs facilitates monoamine-induced IP_3 production, which causes Ca^{2+} release from an internal store, which in turn induces the LTP. To our knowledge, this has been the only example implicating the role of postsynaptic $GABA_B$Rs in synaptic plasticity. In Purkinje neurons, $GABA_B$R activation reduces the Ca^{2+} influx through P-type Ca^{2+} channels (Mintz and Bean, 1993). This mechanism potentially contributes to the suppression of RP, because the increase in intracellular Ca^{2+} concentration is involved in RP. Another signaling cascade possibly implicated in the suppression of RP and downstream to $GABA_B$R activation is the inhibition of adenylyl cyclase through the G_i/G_o proteins. Kano and Konnerth (1992) demonstrated that GABA responsiveness is increased by activation of cAMP-dependent protein kinase (PKA). Thus, PKA activity might be involved in RP and the postsynaptic $GABA_B$R activity may inhibit this pathway. We have examined the downstream signaling cascade of $GABA_B$R activation and present data suggesting the involvement of G_i/G_o proteins, cAMP, and PKA in the suppression of RP.

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Results

Suppression of RP by Presynaptic Activation

We performed simultaneous paired whole-cell patch-clamp recordings from a presynaptic IN and a postsynaptic PN in cerebellar cultures. The membrane potential of an IN was controlled under either a current-clamp or a voltage-clamp condition (at −70mV), whereas that of a PN was kept at −70 mV under a voltage-clamp condition. An inhibitory postsynaptic current (IPSC) was recorded from a PN when an IN was stimulated by positive current injection (under a current-clamp condition) or by a short depolarization pulse (under a voltage-clamp condition) large enough to generate an action potential or inward Na^+ current, respectively (Figures 1A and 1B). The mean amplitude, rise time, and half decay time of evoked IPSCs used for experiments were 236 ± 220 pA, 7.6 ± 2.2 ms, and 14.3 ± 5.6 ms (16 cells), respectively. The conditioning depolarizations (five 500 ms pulses to 0 mV at 0.5 Hz) of a PN potentiated the IPSC for more than 30 min (232% ± 44%, mean ± SEM, 20 min after the conditioning) as previously reported (Kano et al., 1992; Hashimoto et al.,1996) (Figures 1C and 1F). We examined the effect of presynaptic activity on RP induction by stimulating the IN in conjunction with conditioning depolarizations of the PN. The IN was stimulated nine times at 20 Hz during each 500 ms depolarization. This conjunctive conditioning potentiated the IPSC only slightly, and the IPSC amplitude returned to the basal level 15 min after the conditioning (110% ± 23%, at 20 min) (Figures 1D and 1F). Conditioning stimulation of a presynaptic IN by itself did not affect the IPSC (109% ± 22%, at 20 min) (Figures 1E and 1F). Thus, the RP of inhibitory synaptic transmission between an IN and a PN is suppressed by activating the presynaptic IN during the conditioning depolarizations of the postsynaptic PN. None of the conditionings affected the time course of evoked IPSCs and other recording conditions such as the holding current, input resistance, and series resistance of both pre- and postsynaptic neurons (data not shown).

RP Is Expressed as $GABA_A$R Potentiation and Is Suppressed by $GABA_B$R Activation

It has been reported that RP is accompanied by an increased responsiveness of $GABA_A$Rs on a PN (Kano et al., 1992). To monitor the $GABA_A$ responsiveness, we iontophoretically applied GABA or muscimol (a selective $GABA_A$R agonist) to a PN through a glass pipette aimed at a primary or secondary dendrite in the presence of tetrodotoxin (TTX, 1 μM) and 6-Cyano-7-nitroquinoxaline-2, 3-dione disodium (CNQX, 10 μM), inhibitors, respectively, of voltage-gated Na^+ channels and ionotropic glutamate receptors of non-N-methyl-D-aspartate (NMDA) type. The conditioning depolarizations of a PN potentiated the GABA or muscimol response for more than 30 min (174% ± 18% and 170% ± 12%, respectively, at 20 min) (Figures 2A, 2C, and 2D). During the conditioning, neither GABA nor muscimol was applied. GABA and muscimol response were completely blocked by bicuculline (20 μM), a selective antagonist for $GABA_A$Rs (data not shown). We then confirmed that the $GABA_A$R responsiveness is potentiated by the depolarizations of a PN.

We next addressed how presynaptic activation suppressed RP. Since GABA is released from the presynaptic terminals, GABA is an obvious candidate for the mediator of suppressive effect on RP. To examine the effect of GABA on RP, we coupled iontophoretic application of GABA with the conditioning depolarizations of a PN. GABA was applied once during each 500 ms depolarizing pulse. This conjunctive conditioning potentiated the GABA response only slightly and its amplitude returned to the initial level 15–20 min after the conditioning (104% ± 8%, at 20 min) (Figures 2B and 2C). Therefore, we conclude that GABA exerts a suppressive effect on RP. On the other hand, iontophoretic muscimol application coupled with the depolarizations had no effect on the RP amplitude (176% ± 22%, at 20 min) (Figure 2D).

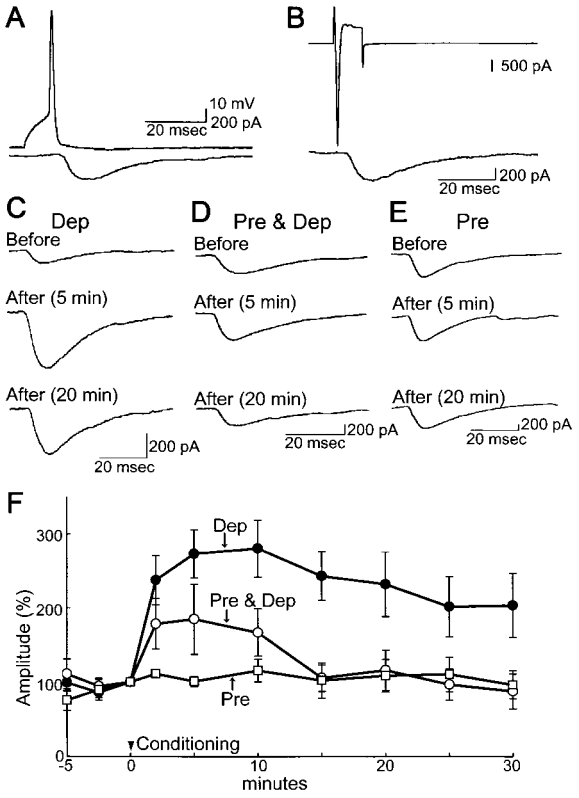

Figure 1. RP of Evoked IPSC and Its Suppression by Activation of the Presynaptic Neuron

(A) Sample traces of a presynaptic action potential and the evoked IPSC in a PN. IPSCs were recorded as inward currents, since the internal solution contained a high concentration of Cl^-.

(B) Sample traces of a presynaptic inward current induced by 10 ms voltage pulse to −40 mV and the evoked IPSC in a PN.

(C–E) Representative traces of averaged IPSCs (n = 5) before and after the conditioning stimulations.

(C) Dep, depolarizations of a PN were applied as a conditioning stimulation.

(D) Pre & Dep, depolarizations of a PN coupled with repetitive stimulation of a presynaptic neuron were applied as a conditioning stimulus.

(E) Pre, only a presynaptic neuron was repetitively stimulated as a conditioning.

(F) The time courses of IPSC amplitudes before and after conditioning stimulations applied at 0 min. Data presented are mean ± SEM from six independent trials for Pre & Dep together and from five trials each for Dep and Pre independently. In each trial, amplitudes of IPSCs were normalized taking the mean value between −2 min and 0 min as 100%. Other points in the graph represent data obtained between ±2 min of the indicated times. Significant differences ($p < 0.05$, Student's t test) were detected between Dep and Pre after 0 min and between Dep and Pre & Dep after 5 min.

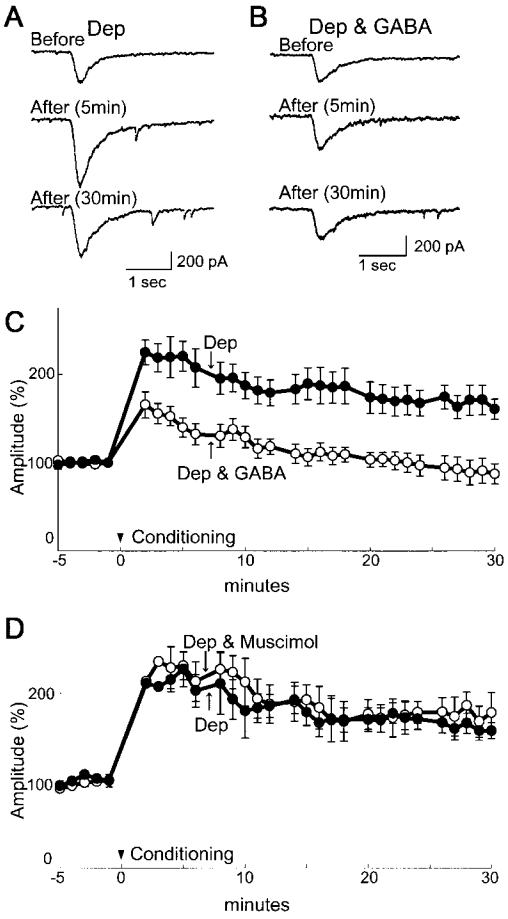

Figure 2. RP of GABA or Muscimol Response and Its Suppression by GABA Application Coupled with Conditioning Depolarizations

(A and B) Representative GABA responses before and after the conditioning depolarizations without (A) or with (B) the coupled GABA application.

(C) The time courses of GABA response amplitude before and after the conditioning depolarizations without (Dep) or with (Dep & GABA) the coupled GABA application (applied at 0 min). The data presented are mean ± SEM from six independent trials for each condition. Significant differences ($p < 0.05$, Student's t test) were detected between Dep and Dep & GABA after the conditioning.

(D) The time courses of muscimol response amplitude before and after the conditioning depolarizations without (Dep, n = 5) or with (Dep & Muscimol, n = 5) the coupled muscimol application. In each trial, amplitudes of GABA or muscimol response were normalized taking the amplitude at −1 min as 100%.

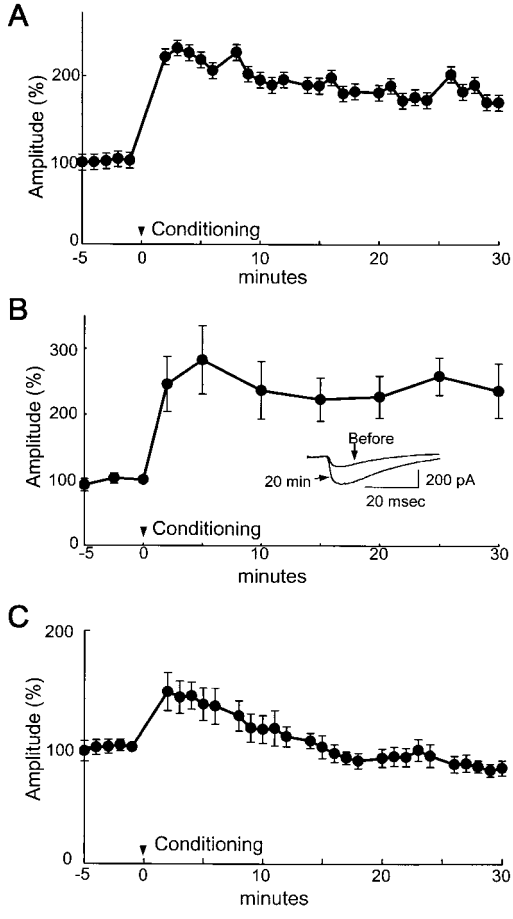

Figure 3. Suppression of RP Is Mediated by $GABA_BR$ Activation

(A and B) Effect of SCH50911 on the suppression of RP.

(A) The time course of amplitude of GABA response amplitude before and after the conditioning depolarizations coupled with GABA application in the presence of SCH50911 (n = 5).

(B) The time course of evoked IPSC amplitude before and after the conditioning depolarizations coupled with presynaptic activation in the presence of SCH50911 (n = 5). The inset shows representative traces of averaged IPSCs (five IPSCs for each) recorded from a PN before and 20 min after the conditioning.

(C) Effect of baclofen on the time course of muscimol response amplitude before and after the conditioning depolarizations (n = 5).

These results suggest that GABA receptors other than $GABA_ARs$ mediate the suppression of RP.

It has been reported that PNs express metabotropic $GABA_BRs$ (Batchelor and Gartwaite, 1992; Kaupmann et al., 1997, 1998; Jones et al., 1998; Kuner et al., 1999). We assumed that $GABA_BRs$ mediate the suppressive effect on RP and examined the effect of a $GABA_BR$ antagonist, (+)-5, 5-dimetyl-2-morpholineacetic acid hydrochloride (SCH50911, 10 μM) (Bolser et al., 1995). In the presence of SCH50911, GABA application coupled with the conditioning depolarizations failed to suppress the RP of GABA response (181% ± 7%, at 20 min) (Figure 3A). Furthermore, SCH50911 prevented the suppression of the RP of evoked IPSC after the conditioning activation of a presynaptic IN coupled with the postsynaptic depolarizations (227% ± 32%, at 20 min) (Figure 3B). These results suggest that $GABA_BR$ activation is required to suppress RP. To confirm this point, we examined the effect of a $GABA_BR$ agonist on RP induction. In the presence of baclofen (20 μM), a selective agonist for $GABA_BRs$, the conditioning depolarizations of a PN failed to induce the RP of muscimol response (91% ± 8%, at 20 min) (Figure 3C). We conclude that $GABA_BR$ is involved in the suppression of RP. When baclofen was applied 10 min after the conditioning depolarizations, the potentiated muscimol response did not return to the initial level (data not shown). This result suggests that only $GABA_BR$ activation during or close to depolarizations suppresses RP.

We also monitored $GABA_AR$ responsiveness by recording miniature IPSCs (mIPSCs) in the presence of TTX and CNQX. A mIPSC is a response to a single synaptic vesicle and is completely blocked by bicuculline. Therefore, the mean amplitude of mIPSCs should

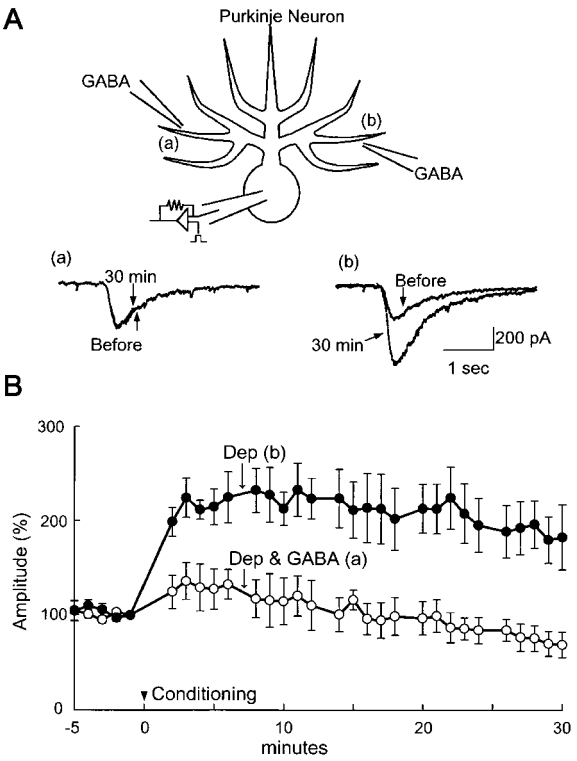

Figure 4. Site-Specific Suppression of RP

(A) GABA was iontophoretically applied to two spatially distinct dendritic sites of a PN (a and b). Representative GABA response traces recorded from each site before and 30 min after the conditioning depolarizations. During the conditioning depolarizations, GABA was applied only to the site a.

(B) The time courses of GABA response amplitudes before and after the conditioning depolarizations recorded from sites a and b. The data presented are mean ± SEM from five PNs. Significant differences ($p < 0.05$, Student's t test) were detected after the conditioning.

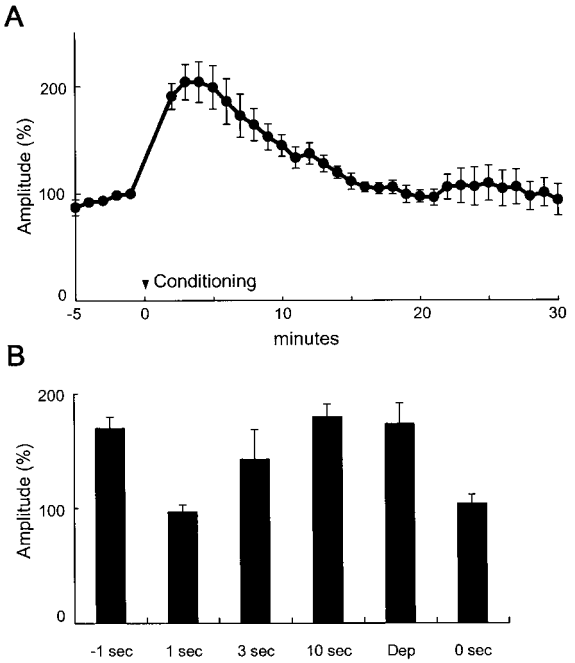

Figure 5. Effective Timing of GABA Application for Suppression of RP

(A) The time course of GABA response amplitude before and after the conditioning in which repetitive GABA application was started 1 s after the end of postsynaptic depolarizations.

(B) The critical timing for conditioning GABA application to suppress RP. The conditioning GABA application was given before or after the conditioning depolarizations. In the former GABA application ended 1 s before the depolarizations (−1 s, n = 5), and in the latter GABA application started 1 s (n = 5), 3 s (n = 5), or 10 s (n = 5) after the end of depolarizations. Dep (n = 6) and 0 s (n = 6) are the data without the conditioning GABA application and with the simultaneous GABA application, respectively. GABA response amplitudes at 20 min after the conditioning depolarizations are presented. Significant differences ($p < 0.05$, Student's t test) were detected between Dep and 0 s or 1 s.

also reflect the sensitivity of $GABA_A$Rs on a PN. Conditioning depolarization of a PN potentiated the mean amplitude of mIPSCs for more than 30 min (165% ± 21% [n = 7], at 20 min) (data not shown). In the presence of baclofen, the RP of mIPSCs was suppressed (99% ± 6% [n = 7], at 20 min), whereas in the presence of SCH50911 the RP of mIPSCs was slightly larger (203% ± 18% [n = 5], at 20 min) than that in its absence (no significant difference). We conclude from the above that the postsynaptic $GABA_B$Rs activated by GABA released from presynaptic terminals during the postsynaptic depolarizations suppresses RP of $GABA_A$ responsiveness. Application of baclofen affected neither the amplitude and time course of mIPSCs, nor the responses to muscimol and recording conditions.

Site Specificity of RP Suppression

RP is not a synapse-specific mechanism. It should be induced at all inhibitory synapses on a PN after strong depolarizations. In contrast, the suppression of RP can be regulated at individual synapses because it is brought about by presynaptic activity. To examine whether RP is suppressed only at the site that receives GABA, we iontophoretically applied GABA to two spatially distinct regions of PN dendrites (>100 μm apart) (Figure 4A). GABA responses were monitored by applying GABA alternately to each site every 30 s. During the conditioning depolarizations, GABA was applied only to site a, not to site b. The RP of GABA response was suppressed only at site a (97% ± 18%, at 20 min); whereas at site b, the RP was induced (213% ± 26%, at 20 min) (Figure 4). The result that RP induction was suppressed only at the site where GABA was applied during the conditioning depolarizations indicates that the RP suppression is site specific and suggests a possible mechanism for synapse-specific regulation of transmission efficacy.

Effective Timing of GABA Application to Suppress RP

To examine the critical time window for GABA application to suppress RP, we changed the timing of iontophoretic GABA application relative to the conditioning depolarizations of a PN. When the conditioning GABA application (five times at 0.5 Hz) ended 1 s before the start of conditioning depolarizations, the RP of GABA response was not suppressed (170% ± 10%, at 20 min) (Figure 5B). In contrast, when the conditioning GABA application was started 1 s after the end of depolarizations, the RP was suppressed (97% ± 6%, at 20 min)

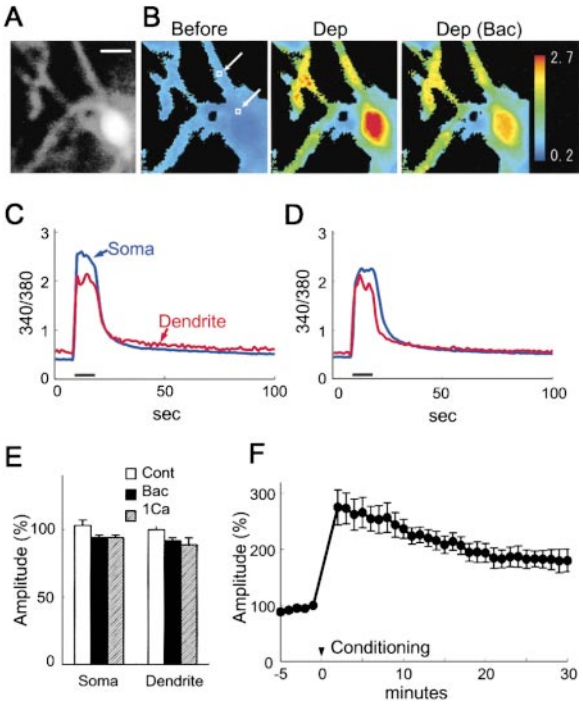

Figure 6. $GABA_BR$-Mediated Reduction in Intracellular Ca^{2+} Increase and Effect on RP

(A) The fluorescence image (380 nm) of a PN loaded with fura-2. The scale bar indicates 20 μm.

(B) The fluorescence ratio images (340/380) of a PN before and during the conditioning depolarizations without (Dep) and with baclofen [Dep (Bac)].

(C and D) The time course of the fluorescence ratios in a soma and a dendrite (indicated by arrows in [B]) without (C) or with (D) baclofen. The conditioning depolarizations (indicated by a bar) started at 10 s.

(E) The peak amplitude of fluorescence ratio in a soma and a dendrite during the conditioning depolarizations without (Cont, n = 5) or with baclofen (Bac, n = 8) and in the low Ca^{2+} solution (1Ca, n = 6).

(F) The time course of GABA response amplitude before and after the conditioning depolarizations in the low Ca^{2+} solution (n = 5).

except for a transient potentiation that was larger than that observed after the conditioning depolarizations accompanied by the simultaneous GABA application (Figures 5A and 5B). The conditioning GABA application started 3 s after the depolarizations slightly suppressed the RP (143% ± 26%, at 20 min), but that started 10 s after the depolarizations failed to suppress the RP (180% ± 11%, at 20 min) (Figure 5B). These results indicate that $GABA_BR$ activation during or within 1 s after the conditioning depolarizations is sufficient to suppress the RP.

Ca^{2+} Imaging

Mintz and Bean (1993) reported that $GABA_BR$ activation reduces Ca^{2+} current through P-type Ca^{2+} channels in a PN. To examine whether this effect causes the suppression of RP, we measured the intracellular Ca^{2+} concentration in a PN using fura-2 (Figure 6A). The conditioning depolarizations increased the Ca^{2+} concentration in both a soma and dendrites (Figures 6B and 6C). Baclofen slightly reduced the depolarization-induced increase in intracellular Ca^{2+} concentration (Figures 6B–6D). The peak amplitudes of the fluorescence ratios (the fluorescence excited at 340 nm divided by that excited at 380 nm) in the soma and in the proximal or secondary dendrites after baclofen application were 94% ± 2% and 92% ± 2%, respectively (Figure 6E). To examine whether such a slight reduction in Ca^{2+} concentration increase contributes to the suppression of RP, we searched for another condition in which a comparable or slightly larger reduction of the depolarization-induced increase in intracellular Ca^{2+} concentration takes place. Decreasing the external Ca^{2+} concentration to 1 mM reduced the depolarization-induced increase in Ca^{2+} concentration to 89% ± 5% in the dendrites (Figure 6E). Even in this low Ca^{2+} external solution, the conditioning depolarizations induced the RP of GABA response (194% ± 17%, at 20 min) (Figure 6F). These results together with the data that the conditioning GABA application 1 s after the depolarizations suppressed RP suggest that the main signaling cascade between the $GABA_BR$ activation and the suppression of RP is other than the reduction of Ca^{2+} influx through voltage-gated Ca^{2+} channels.

Involvement of G_i/G_o, cAMP, and PKA in Suppression of RP

It has been reported that $GABA_BR$ is coupled to the G_i/G_o proteins that inactivate adenylyl cyclases (Misgeld et al., 1995; Kaupmann et al., 1997; Kuner et al., 1999). To address whether the $GABA_BR$-mediated suppression of RP is G_i/G_o dependent, we examined the effect of G protein inhibitors on the suppression of RP. When $GDP_\beta S$ (500 μM), an inhibitor for G proteins, was present in the patch pipette, the conditioning GABA application coupled with the depolarizations failed to suppress the RP of GABA response (180% ± 20%, at 20 min) (Figure 7B). After cultured PNs were pretreated overnight with pertussis toxin (PTX, 500 ng/ml), an inhibitor of G_i/G_o proteins, the GABA application coupled with the conditioning depolarizations also failed to suppress the RP (179% ± 12%, at 20 min) (Figures 7A and 7B). Furthermore, N-ethylmaleimide (NEM, 10 μM, applied to bath 5 min before the conditioning), an inhibitor of PTX-sensitive G proteins, also prevented the suppression of RP (168% ± 12%, at 20 min) (Figure 7B). These results indicate that PTX-sensitive G_i/G_o proteins are involved in the suppression of RP.

Activation of the G_i/G_o protein decreases the intracellular cAMP concentration through inhibition of adenylyl cyclases, which should decrease PKA activity. We next investigated whether or not cAMP and PKA are involved in the suppression of RP. When the PKA activity was raised by pretreating cultured neurons with Sp-cAMPS-AM (10 μM), a PKA activator, the GABA application coupled with the conditioning depolarizations failed to suppress the RP of GABA response (167% ± 7%, at 20 min) (Figures 7C and 7D). Forskolin (50 μM, applied to the bath 5 min before the start of recordings), an activator of adenylyl cyclases, also prevented the RP suppression by GABA application coupled with the depolarizations (182% ± 27%, at 20 min) (Figure 7D). These results suggested that a decrease in PKA activity is involved in the suppression of RP. To examine this issue further, we applied the conditioning depolarizations in the presence of PKA inhibitors. KT5720 (10 μM), a specific inhibitor of PKA, suppressed the RP of GABA response after the conditioning depolarizations (108% ± 14%, at 20 min) (Figures 7E and 7F), suggesting that PKA activity

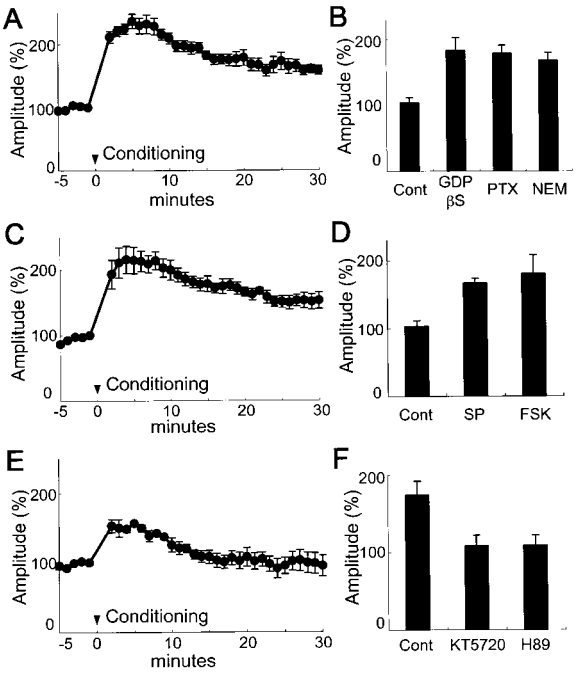

Figure 7. Involvement of G_i/G_o, cAMP, and PKA in Suppression of RP

(A) The time course of GABA response amplitude before and after the conditioning depolarizations coupled with GABA application after PTX treatment (n = 5).

(B) Effects of GDP-$_\beta$S, PTX, and NEM on the suppression of RP. The GABA response amplitudes 20 min after the conditioning depolarizations coupled with GABA application are presented (n = 5 for each). Significant differences ($p < 0.01$, ANOVA and post-hoc test, Fisher's PLSD) were detected between Cont and GDP$_\beta$S, PTX, or NEM.

(C and D) PKA activation abolished the suppression of RP.

(C) The time course of GABA response amplitude before and after the conditioning depolarizations coupled with GABA application in PNs pretreated with Sp-cAMPS-AM (n = 5).

(D) Effects of Sp-cAMPS-AM (SP) and forskolin (FSK) on the suppression of RP. The GABA response amplitudes 20 min after the conditioning depolarizations coupled with GABA application are presented (n = 5 for each). Significant differences ($p < 0.05$, ANOVA and post-hoc test) were detected between Cont and SP or FSK.

(E and F) Suppression of the RP by PKA inhibitors.

(E) The time course of GABA response amplitude before and after the conditioning depolarizations in the presence of KT5720 (n = 5).

(F) The GABA response amplitudes 20 min after the conditioning depolarizations in the absence (Cont) or presence of KT5720 or H89 are presented (n = 5 for each). Significant differences ($p < 0.05$, ANOVA and post-hoc test) were detected between Cont and KT5720 or H89.

is required for RP. H89 (100 nM), an inhibitor specific for PKA at this concentration, also suppressed the RP (109% ± 13%, at 20 min) (Figure 7F). Thus, PKA inhibitors mimicked the GABA application coupled with the conditioning depolarizations of a PN. Application of NEM, Forskolin, KT5720, and H89 to the external solution did not affect the basal GABA response (data not shown). Inclusion of GDP$_\beta$S in the internal solution did not cause recognizable change in the basal GABA response (data not shown). These results suggest that $GABA_BR$ activation decreases the cAMP concentration through activation of G_i/G_o proteins, which reduces the PKA activity and suppresses RP.

Discussion

Suppression of Synaptic Plasticity by Presynaptic Activity

Synaptic plasticities at excitatory glutamatergic synapses have been studied extensively (Ito, 1989; Bliss and Collingridge, 1993; Malenka, 1994; Linden and Connor, 1995; Daniel et al., 1998). At inhibitory GABAergic synapses, long-term and short-term synaptic plasticities have been reported in the cerebral and cerebellar cortex, cerebellar nuclei, hippocampus, and Mauthner neurons in goldfish (Kano, 1995; Marty and Llano, 1995; Komatsu, 1996; Aizenman et al., 1998; Nusser et al., 1998; Oda et al., 1998). Most synaptic plasticities at both glutamatergic and GABAergic synapses are induced by repetitive activation of presynaptic neurons. This use-dependent induction mechanism enables synapse-specific regulation of the transmission efficacy. On the other hand, at cerebellar inhibitory synapses between an IN and a PN, the postsynaptic depolarization caused by heterosynaptic climbing fiber activation induces RP without the homosynaptic activation (Kano et al., 1992). There, the transmission efficacy at a number of synapses is regulated simultaneously. We have demonstrated that presynaptic activation in conjunction with the postsynaptic depolarizations suppresses RP and have also shown that the suppression of RP is site specific. This suggests that the suppression of RP provides a synapse-specific way to regulate the transmission efficacy. It seems that depolarization of a PN that can be caused by heterosynaptic climbing fiber activation induces RP not at the inhibitory synapses that were active during the postsynaptic depolarizations, but at the synapses that were inactive during the depolarizations. This suppression of RP is a novel form of regulation of synaptic plasticity by presynaptic activity.

Physiological Roles of RP and Its Suppression

In paired recordings, presynaptic activation at 20 Hz coupled with conditioning depolarizations suppressed RP. In our cultures, the spontaneous firing frequency of an IN is usually several Hz, and an IN occasionally shows bursts of action potentials (>20 Hz) lasting longer than 500 ms, when examined in cell-attached patch-clamp recording mode (data not shown). A previous study on slice preparation showed that the spontaneous firing frequency of an IN sometimes reaches 40 Hz (Llano and Marty, 1995). Therefore, the suppression of RP by presynaptic activity seems to occur under physiological conditions. We also observed that the RP of mIPSCs tended to be more pronounced in the presence of SCH50911, which might suggest that $GABA_BR$ activation by spontaneously released GABA suppresses RP at some inhibitory synapses. Previously, Kano et al. (1992) reported that the RP of spontaneous IPSCs sometimes attenuated to the basal level within 15 min after conditioning depolarizations in slice preparations. This might be also caused by the spontaneous activity of presynaptic INs. All these suggest that the suppression of RP works in vivo, where INs could be more active. Considering the relatively high firing frequency of INs, synaptic efficacy at IN–PN synapses could be even regulated as follows. Initially RP might be suppressed at some inhibitory synapses on a PN due to high resting activities of presynaptic INs, so that RP is triggered by selective silencing of these INs. In other words, RP would be

released from the suppression by downregulation of the presynaptic IN activities. In any case, we suggest that the interplay of postsynaptic PN activity and presynaptic IN activity determines the synaptic efficacy at IN–PN synapses.

In the cerebellum, LTD at the glutamatergic synapses between parallel fibers and a PN has been proposed as a cellular basis for motor learning (Aiba et al., 1994; Conquet et at., 1994; Kashiwabuchi et al., 1995; De Zeeuw et al., 1998; also see Ito, 1989; Lisberger, 1998). The LTD is induced by repetitive activation of parallel fibers in conjunction with climbing fiber activation (Ito, 1989; Linden and Connor, 1995; Daniel et al., 1998). Climbing fiber activation also contributes to RP (Kano et al., 1992). The functional significance of RP has not been explored in vivo. RP might work to suppress overexcitation of a PN by enhancing inhibition when a PN has been strongly depolarized repetitively, and its suppression might control the level of inhibition by integrating the activity of each synaptic input during the depolarizations. Thus, the suppression of RP might provide fine control of activity level of a PN by integrating both pre- and postsynaptic activities and contribute to the refined performance of the cerebellar cortical network. In a computational model study, it has been proposed that inhibitory inputs to a PN are more influential than excitatory parallel fiber inputs in controlling the firing pattern of action potentials in a PN (Jaeger and Bower, 1999). Thus, in coordination with LTD, RP and its synapse-specific suppression by presynaptic activity might play a critical role in information processing and storage in the cerebellum.

Molecular Mechanism of Suppression of RP

We confirmed that RP is accompanied by increased responsiveness of $GABA_A$Rs on a PN. The extents of potentiation of evoked IPSC, mIPSC, and GABA or muscimol response were comparable (≈200%) as shown here, which suggests that RP is expressed exclusively postsynaptically. However, the presynaptic contribution to the RP of evoked IPSC is undeniable. At the IN–PN synapses, short-term modulation of the transmission ascribed to the presynaptic change has been reported (Llano et al., 1991; Vincent et al., 1992), and the existence of a retrograde messenger at the synapses controlling the presynaptic release probability has been suggested (Glitsch et al., 1996).

The cerebellar PNs express $GABA_B$R1 and $GABA_B$R2 (Kaupmann et al., 1997, 1998; Jones et al., 1998; Kuner et al., 1999). We have also demonstrated that postsynaptic $GABA_B$Rs are involved in the suppression of RP. This is the first demonstration of the role of postsynaptic $GABA_B$Rs in the suppression of synaptic plasticity. Previously, two examples implicating the role of $GABA_B$Rs in synaptic plasticity have been reported. One is LTP in the hippocampus (Davies et al., 1991; Mott and Lewis, 1991). Presynaptic $GABA_B$Rs facilitate the induction of LTP in the CA1 region. GABA released by repetitive stimulation activates presynaptic $GABA_B$Rs, which suppress GABA release and contributes to the postsynaptic depolarization caused by excitatory glutamatergic inputs. The other is LTP at inhibitory synapses in the visual cortex (Komatsu, 1996). The activation of postsynaptic $GABA_B$Rs facilitates monoamine-induced IP_3 formation causing Ca^{2+} release from an internal store, which then triggers the LTP. It is intriguing that activation of $GABA_B$Rs in a PN suppressed only the potentiation of $GABA_A$ responsiveness and affected neither the basal response nor the potentiated response. Thus, the $GABA_B$R activation seemed to affect only the cascade inducing RP but did not regulate the $GABA_A$Rs directly by itself.

We have explored the downstream signaling cascade of $GABA_B$R activation to suppress RP. It has been reported that the increase in intracellular Ca^{2+} and subsequent activation of CaMK II is required for RP induction (Kano et al., 1992, 1996; Hashimoto et al., 1996). Mintz and Bean (1993) reported that the Ca^{2+} current through P-type channels is inhibited by the $GABA_B$R activation in a PN. This $GABA_B$R-mediated reduction of Ca^{2+} influx could contribute to the suppression of RP. However, our Ca^{2+} imaging experiment demonstrated that $GABA_B$R-mediated reduction of the depolarization-induced increase in intracellular Ca^{2+} is marginal. We have also shown that the comparable reduction of intracellular Ca^{2+} increase by decreasing external Ca^{2+} concentration does not affect RP induction. These results suggest that a signaling cascade other than Ca^{2+} is implicated in the suppression of RP.

Kano and Konnerth (1992) showed that administration of a PKA activator, 8-br-cAMP, potentiates the GABA response in a PN. As shown in Figure 7, RP is inhibited by PKA inhibitors, suggesting that PKA is required for RP induction. Thus, both PKA and CaMK II seem to be implicated in RP. We have demonstrated that inhibition of the G_i/G_o proteins or activation of PKA inhibited the suppression of RP. These results suggest that the $GABA_B$R-dependent suppression of RP is mediated by the G_i/G_o activation, decrease in intracellular cAMP concentration, and downregulation of PKA activity. Previous studies (Chen et al., 1990; Krishek et al., 1994; Yamada and Akasu, 1996; McDonald et al., 1998; also see Smart, 1997) revealed that various kinds of protein kinases modify the function of $GABA_A$Rs. Whether or not RP is due to direct phosphorylation of $GABA_A$Rs is to be elucidated.

Experimental Procedures

Culture

Methods of preparing primary cultures of cerebellar neurons from Wister rats were similar to those described previously (Hirano and Kasono, 1993). Briefly, cerebella were dissected out from ~18-day-old fetuses, and their meninges were removed. The cerebella were incubated in Ca^{2+} and Mg^{2+}-free Hank's balanced salt solution containing 0.1% trypsin and 0.05% DNase for 15 min at 37°C. Neurons were then dissociated by trituration and cultured in a defined medium for more than 4 weeks. Half of the culture medium was changed every 4 days.

Electrophysiology

Whole-cell patch clamp recordings from cerebellar neurons grown in culture for ~4–6 weeks were performed in the solution containing 145 mM NaCl, 5 mM KOH, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose (pH 7.3) at room temperature (~20°C–24°C). The external solution contained 10 μM CNQX (Tocris) to suppress glutamatergic EPSCs. Neurons were identified as described previously (Hirano and Kasono, 1993). Patch pipettes used to record from PNs were filled with an internal solution (pH 7.3) containing 150 mM CsCl, 15 mM CsOH, 0.5 mM Ethylene glycol bis (β-aminoethylether) N,N,N',N'-tetraacetic acid (EGTA), 10 mM HEPES, 2 mM Mg-ATP (Sigma), and 0.2 mM Na-GTP (Sigma). Mg-ATP and Na-GTP were used to minimize the rundown of $GABA_A$ responsiveness. The electrode resistance was ~3–5 MΩ. In paired recordings, a presynaptic neuron was recorded with an ~5–6 MΩ patch pipette containing 147 mM KCl, 5 mM EGTA, 10 mM HEPES, 15 mM KOH

(pH 7.3). The membrane potential of PNs were held at −70 mV unless otherwise stated. Junction potentials were offset. At this potential and with the internal solution containing 150 mM Cl^-, the IPSCs and GABA responses were recorded as inward currents. The evoked IPSCs were recorded at 0.15 Hz unless otherwise stated. Only recordings with an input resistance of more than 100 MΩ and series resistance of less than 30 MΩ were accepted. Series resistance and input resistance were monitored every 5 min, and experiments were terminated when a change of more than 20% was detected. We selected neuronal pairs in which the amplitude of the IPSC was less than 500 pA. When the IPSC amplitude was larger than 500 pA, we could not suppress dendritic Ca^{2+} spike generation at unclamped regions. About 30% of examined pairs showed the inhibitory synaptic connection. About 20% of PNs of synaptically connected pairs showed an IPSC of appropriate amplitude and were used for the experiments. We succeeded in long-term (<40 min) stable recording of IPSCs in 30% of selected neuronal pairs. Thus, the success rate of prolonged paired recording was about 2%.

The method for iontophoretic application of GABA or muscimol (Research Biochemicals International) was similar to previous studies (Linden et al., 1991; Hirano et al., 1994). A glass pipette containing 10 mM GABA or 300 μM muscimol with 10 mM HEPES was aimed at a proximal or secondary dendrite of a PN, and 20 ms positive voltage pulses were applied every minute. The conditioning depolarizations of a PN were five 500 ms pulses to 0 mV at 0.5 Hz. Presynaptic stimulation coupled with the conditioning depolarizations was applied at 20 Hz during each 500 ms depolarization of a PN. Iontophoretic application of either GABA or muscimol coupled with the depolarizations was given 100 ms after the onset of each 500 ms depolarization pulse. During the uncoupled depolarizations, neither presynaptic stimulation nor $GABA_A$ agonist application was given. In the two-site electrophoresis experiment, GABA was iontophoretically applied through two independent glass pipettes aimed at two dendritic regions of a PN (100 μm < apart), alternately every 30 s (Kasono and Hirano, 1994). During the conditioning depolarizations, GABA was applied to only one site. In the experiments to monitor mIPSCs, GABA, or muscimol responses, 1 μM TTX (Wako, Japan) was used to suppress action potentials. CNQX, TTX, baclofen (Tocris), SCH50911 (Tocris), NEM (Sigma), forskolin (Tocris), KT5720 (Calbiochem), and/or H89 (Calbiochem) were applied to the bath. Sp-cAMPS-AM (Biolog) was loaded to neurons for 30 min and washed before the start of recording. Sp-cAMPS-AM in the external solution immediately suppresses the GABA response and IPSCs. This suppressive effect of Sp-cAMPS-AM on GABA response is likely due to the blocking of $GABA_A$Rs from the outside and seems common to most of cAMP analogs (Lambert and Harrison, 1990). PTX (List Biological Labolatories) treatment of neurons was overnight. GDP-$_\beta$S (Sigma) was substituted for GTP of the internal solution in some experiments.

Ca^{2+} Imaging

The intracellular Ca^{2+} concentration in a PN was measured with a Ca^{2+} imaging system (ARGUS/Hisca, Hamamatsu Photonics, Japan) mounted on an inverted microscope (Olympus IX70, Japan) using fura-2 (Calbiochem) (Konnerth et al., 1992). Fura-2 (200 μM) was loaded into a PN through a patch pipette. A PN was excited alternately at 340 nm and 380 nm for 250 ms, and each fluorescence image was recorded at 1 Hz. After recording, the fluorescence ratio (the fluorescence excited at 340 nm divided by that excited at 380 nm) was calculated. Baclofen (20 μM) or the low Ca^{2+} (1 mM) external solution was applied by perfusion. The peak fluoresence ratio during the depolarizations after solution exchange was compared with that before. In the control experiment, the peak fluorescence ratio 5 min (time required for solution exchange) after the first recording was used. The low Ca^{2+} solution was prepared by replacing 1 mM Ca^{2+} by Mg^{2+} in the external solution.

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